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Short communication

Comparison of the protective efficacy of DNA and baculovirus-derived protein vaccines for EBOLA virus in guinea pigs

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Abstract

The filoviruses Ebola virus (EBOV) and Marburg virus (MARV) cause severe hemorrhagic fever in humans for which no vaccines are available. Previously, a priming dose of a DNA vaccine expressing the glycoprotein (GP) gene of MARV followed by boosting with recombinant baculovirus-derived GP protein was found to confer protective immunity to guinea pigs (Hevey et al., 2001. Vaccine 20, 568–593). To determine whether a similar prime-boost vaccine approach would be effective for EBOV, we generated and characterized recombinant baculoviruses expressing full-length EBOV GP ($GP_{1,2}$) or a terminally-deleted GP (GPa-) and examined their immunogenicity in guinea pigs. As expected, cells infected with the GPa- recombinant secreted more GP_1 than those infected with the $GP_{1,2}$ recombinant. In lectin binding studies, the insect cell culture-derived GPs were found to differ from mammalian cell derived virion GP, in that they had no complex/hybrid N-linked glycans or glycans containing sialic acid. Despite these differences, the baculovirus-derived GPs were able to bind monoclonal antibodies to five distinct epitopes on EBOV GP, indicating that the antigenic structures of the proteins remain intact. As a measure of the ability of the baculovirus-derived proteins to elicit cell-mediated immune responses, we evaluated the T-cell stimulatory capacity of the GPa- protein in cultured human dendritic cells. Increases in cytotoxicity as compared to controls suggest that the baculovirus proteins have the capacity to evoke cell-mediated immune responses. Guinea pigs vaccinated with the baculovirus-derived GPs alone, or in a DNA prime-baculovirus protein boost regimen developed antibody responses as measured by ELISA and plaque reduction neutralization assays; however, incomplete protection was achieved when the proteins were given alone or in combination with DNA vaccines. These data indicate that a vaccine approach that was effective for MARV is not effective for EBOV in guinea pigs.
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Keywords: Filovirus; Ebola virus; Prime-boost; Baculovirus; DNA vaccine

The Filoviridae family contains two genera of highly pathogenic viruses: Ebola-like viruses, and Marburg-like viruses (IUMS, 2000). Currently four viral species are listed in the Ebola-like virus genus: Cote d'Ivoire Ebola virus, Reston Ebola virus (REBOV), Sudan Ebola virus (SEBOV), and Zaire Ebola virus (ZEBOV). Numerous strains of these viruses have been identified. One species of Marburg-like viruses has been officially designated, Marburg virus (MARV), with several strains identified. It is likely that as these strains are better characterized, some of them will be elevated to viral species. All of the viruses in the family except for REBOV are highly pathogenic for humans. ZEBOV caused two major outbreaks of Ebola hemorrhagic fever (EHF) in 1976 and 1995, during which hundreds of cases were reported with mortality rates as high as 80–90% (Peters et al., 1994). SEBOV caused outbreaks in Sudan in 1976 and 1979 with ~300 cases and mortality ~50%. SEBOV appeared in Uganda in 2000–2001 causing ~400 cases of EHF with a mortality rate of ~

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14. ABSTRACT

The filoviruses Ebola virus (EBOV) and Marburg virus (MARV) cause severe hemorrhagic fever in humans for which no vaccines are available. Previously, a priming dose of a DNA vaccine expressing the glycoprotein (GP) gene of MARV followed by boosting with recombinant baculovirus-derived GP protein was found to confer protective immunity to guinea pigs (Hevey et al., 2001. Vaccine 20, 568-593). To determine whether a similar prime-boost vaccine approach would be effective for EBOV, we generated and characterized recombinant baculoviruses expressing full-length EBOV GP (GP(1,2)) or a terminally-deleted GP (GPa-) and examined their immunogenicity in guinea pigs. As expected, cells infected with the GPa- recombinant secreted more GP(1) than those infected with the GP(1,2) recombinant. In lectin binding studies, the insect cell culture-derived GPs were found to differ from mammalian cell derived virion GP, in that they had no complex/hybrid N-linked glycans or glycans containing sialic acid. Despite these differences, the baculovirus-derived GPs were able to bind monoclonal antibodies to five distinct epitopes on EBOV GP, indicating that the antigenic structures of the proteins remain intact. As a measure of the ability of the baculovirus-derived proteins to elicit cell-mediated immune responses, we evaluated the T-cell stimulatory capacity of the GPa- protein in cultured human dendritic cells. Increases in cytotoxicity as compared to controls suggest that the baculovirus proteins have the capacity to evoke cell-mediated immune responses. Guinea pigs vaccinated with the baculovirus-derived GPs alone, or in a DNA prime-baculovirus protein boost regimen developed antibody responses as measured by ELISA and plaque reduction neutralization assays; however, incomplete protection was achieved when the proteins were given alone or in combination with DNA vaccines. These data indicate that a vaccine approach that was effective for MARV is not effective for EBOV in guinea pigs.

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60%. The ecology and epidemiology of EBOV are poorly understood and despite attempts to identify the host species that maintains the virus in nature, it remains unknown (Le Guenno, 1997; Leirs et al., 1999).

EBOV particles consist of helical nucleocapsids containing approximately 19 Kb of single-strand, negative-sense RNA. The viral genome encodes seven structural proteins, one of which, the glycoprotein GP, forms spikes on the surface of the virion. GP is a type 1 membrane protein, and is highly glycosylated with N-linked and O-linked glycans accounting for approximately half of its apparent molecular weight (Feldmann et al., 1994). Maturation of GP involves posttranslational cleavage of a precursor by a furin-like enzyme to yield two fragments, GP_1 and GP_2, which are disulfide-bonded ($GP_{1,2}$) and expressed on the surface of infected cells and virions (Volchkov et al., 1998a). GP_1 is also shed from the surface of infected cells in a soluble form and may contribute to the complex pathogenesis of this virus (Volchkov et al., 1998b).

In addition to mRNA for GP, transcription of the GP gene leads to mRNA encoding a secreted non-structural glycoprotein, sGP (Volchkov et al., 1995). The sGP mRNA is the exact copy of the viral genome, whereas the mRNA of the mature GP is generated by transcriptional editing, which results in the addition of a non-templated adenosine residue to a run of seven adenosine residues (Sanchez et al., 1996). Although the role of sGP is not completely understood, a study using a ZEBOV infectious clone, showed that in the absence of sGP, much of full length GP synthesis is arrested in the endoplasmic reticulum or Golgi (Volchkov et al., 2001). These data suggest that high-level expression of GP overwhelms the transport and glycosylation machinery of the host cell, resulting in increased cytopathology. The presence of sGP may temper the GP expression and cytopathology, allowing the virus to replicate for longer periods of time (Volchkov et al., 2001). It was speculated that sGP might also play a role in pathogenesis by inactivating neutrophils and/or acting as a decoy for anti-EBOV antibodies (Ito et al., 2001; Maruyama et al., 1999a; Yang et al., 1998).

Studies to define important immune responses for protection from EBOV have indicated that both humoral and cell-mediated immunity play critical roles in disease prevention. $GP_{1,2}$ is a target for neutralizing antibodies, which have been shown to be an important element of immunity to EBOV (Jahrling et al., 1996; Mikhailov et al., 1994). Moreover, vaccination with $GP_{1,2}$ offers protection from lethal EBOV challenge in rodent models (Gilligan et al., 1997; Pushko et al., 1997, 2000; Sullivan et al., 2000; Vanderzanden et al., 1998; Xu et al., 1998).

Although a variety of vaccine approaches elicit protective immune responses in rodents, there are currently no effective vaccines or therapies for EBOV infection and EHF in humans. Previous studies on MARV demonstrated that baculovirus-expressed GP offered partial protective immunity to MARV challenge in guinea pigs (Hevey et al., 1997), and that complete protection could be achieved if a priming dose of a DNA vaccine expressing MARV GP was given prior to boosts with the baculovirus expression products (Hevey et al., 2001). In this study, we sought to determine if a similar prime-boost vaccine approach would be effective for EBOV. To answer this question, we first prepared recombinant baculoviruses expressing full-length ZEBOV $GP_{1,2}$ or a truncated version of GP from which the carboxy-terminal anchor region of GP_2 is deleted (GPa-). The full-length GP ($GP_{1,2}$) gene contains eight adenosine residues at the editing site, to ensure production of full length GP (Vanderzanden et al., 1998). The truncated GP gene was constructed by introducing a stop codon at amino acid position 651 of GP. Both genes were inserted into the plasmid transfer vector pAcUW51 (BD Biosciences), behind the polyhedrin promoter and recombinant baculoviruses were generated by homologous recombination with linear baculovirus DNA as described earlier(Schmaljohn et al., 1983).The GPa- gene is 78 nucleotides shorter than full length $GP_{1,2}$ and encodes a protein that is 26 amino acids smaller. Analysis of the expression products of our two recombinant baculoviruses by immune-precipitation and SDS-PAGE revealed that under non-reducing conditions both the full-length $GP_{1,2}$ and the truncated GPa- appear as single bands (−2ME, Fig. 1). Under reducing conditions, GP_1 and GP_2 were readily observed in lysates (not shown) or supernatants of cells infected with the $GP_{1,2}$ recombinant (+2ME, Fig. 1A). Although GP_1 was also apparent in samples from cells infected with the GPa- recombinant, little of the truncated GP_2 expression product was detected in the infected cells (not shown) or the supernatants (Fig. 1A). An earlier study using a vaccinia T7 polymerase system, revealed that a similarly truncated ZEBOV $GP_{1,2}$ gene produced secreted, soluble, disulfide-linked $GP_{1,2}$, whereas full-length $GP_{1,2}$ shed predominantly soluble GP_1 into infected cell supernatants (Volchkov et al., 1998b). In that study, the truncated GP_2 was readily detected in the mammalian cell supernatants by immune precipitation. We suspect that our differing results with regard to secreted, truncated GP_2 relate to differences in the ability of the antibodies to recognize the truncated GP_2 proteins, and/or to glycosylation or other processing differences inherent to insect cell propagation that would result in inefficient complexing of our GP_1 and GP_2. Poor complexing would influence the amount of GP_2 that we could detect if our polyclonal antibody does not specifically recognize GP_2, but rather predominantly recognizes epitopes on GP_1. This supposition is supported by Western blot results in which we were unable to detect GP_2 in supernatants of insect cells

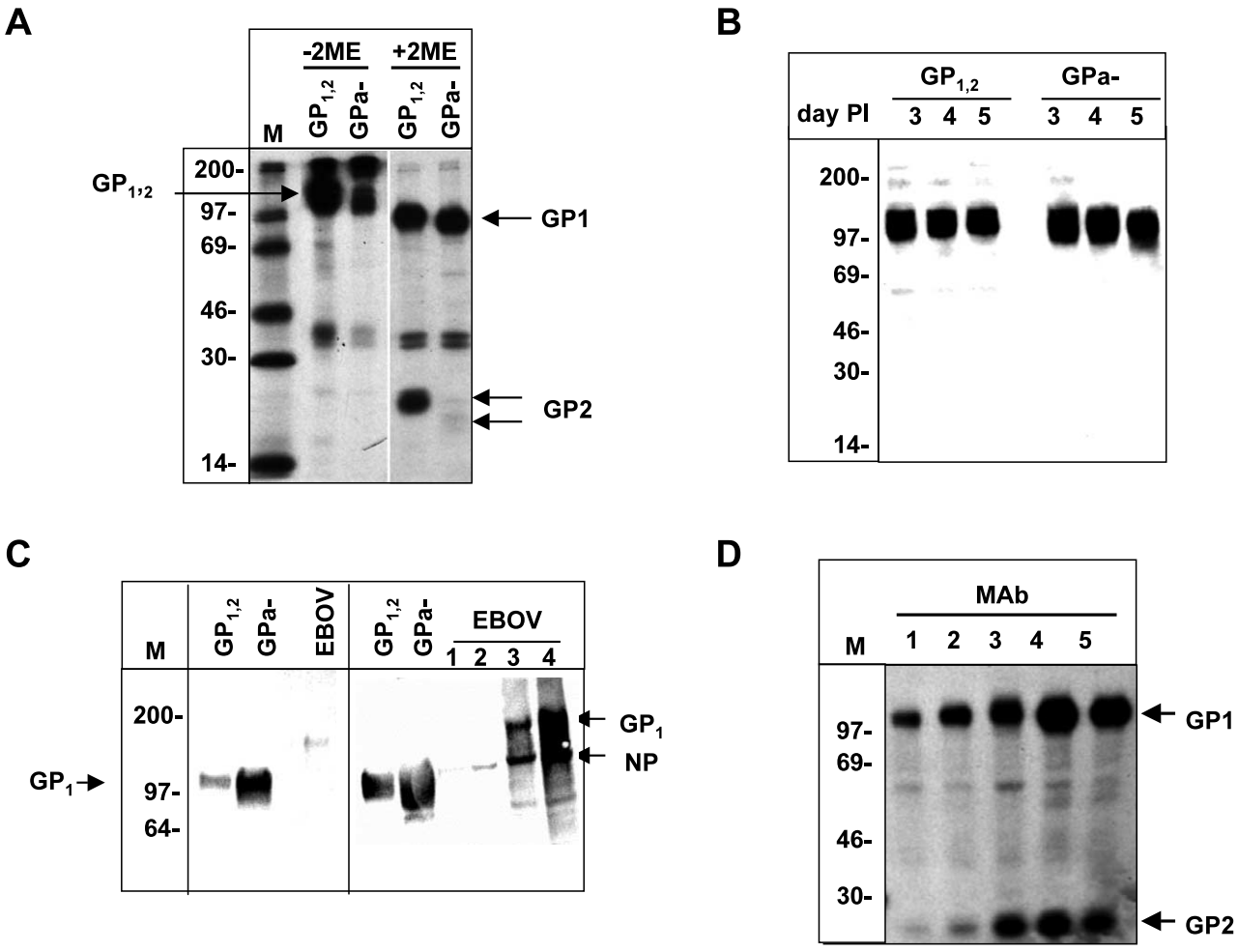

Fig. 1. (A) SDS PAGE of $GP_{1,2}$ and GPa- expression products from supernatants of recombinant baculovirus-infected insect cells radiolabeled from 30 to 42 h after infection with [^{35}S]cysteine and [^{35}S]methionine and immune-precipitated with anti-EBOV guinea pig sera. The positions of the disulfide bonded $GP_{1,2}$ and the individual GP_1 and GP_2 proteins are indicated with arrows. A radiolabeled protein molecular weight marker (M) was included on the gel and the apparent molecular weights are indicated in kDa. (B) Immunoblot detection of GP_1 secreted into the medium of insect cells at days 3, 4, or 5 after infection with the recombinant baculovirues. Aliquots of recombinant baculovirus-infected insect cell supernatants were electrophoresed on SDS-PAGE, then and were electrophoretically transferred to membranes and probed with a polyclonal hyperimmune guinea pig serum. (C) Immunoblot comparison of EBOV virion proteins to baculovirus-derived $GP_{1,2}$ and GPa- products. Twenty liter suspension cultures of *Sf9* cells were infected with either the $GP_{1,2}$ or GPa- baculovirus recombinants and supernatants were collected 48 h later. Aliquots of the supernatants were concentrated by ultrafiltration and were compared to sucrose gradient-purified EBOV by SDS-PAGE and Western blot using a polyclonal anti-EBOV hyperimmune guinea pig serum (left panel) or anti-EBOV monospecific guinea pig sera to GP (right panel). Increasing concentrations of purified EBOV were used in, lanes 1–4 (left panel). GP_1 and nucleocapsid (NP, in virion preparations) are indicated with arrows. (D) Immune precipitation and SDS-PAGE analysis of baculovirus-derived, radiolabeled EBOV $GP_{1,2}$ by using five distinct monoclonal antibodies to EBOV GP.

infected with either the $GP_{1,2}$ or GPa- recombinant virus (Fig. 1B). The two bands that migrate between GP_1 and GP_2 (Fig. 1A) were non-specifically precipitated from cell supernatants when testing other baculovirus recombinants, including a construct expressing EBOV NP using the same polyclonal antibody (data not shown). Because the GP_1 proteins of the two recombinants should be processed identically, we expect that they will have the same properties, including the same size as determined by gel electrophoresis. The apparent slight migration difference of the GP1 proteins derived from the two recombinants and immune precipitated (Fig. 1A) may be due to differences in the amount of protein on the gel, as no migration differences are seen when the proteins are assayed by Western blot (Fig. 1B and C).

Comparing the EBOV proteins present in cell supernatants by Western blot (Fig. 1B) revealed that approximately fourfold more GP_1 was secreted from cells infected with the GPa- recombinant as compared those infected with the $GP_{1,2}$ recombinant as determined by densitometry. We theorized that the size differences of the baculovirus insect cell culture-derived proteins and authentic EBOV proteins observed by Western blot could be due to glycosylation differences. Insect cells are

known to have limited capability to process complex carbohydrates (Jarvis and Finn, 1995) and earlier studies with recombinant baculoviruses expressing MARV GP showed differences in glycosylation between authentic and baculovirus-derived GP as determined by lectin binding assays (Hevey et al., 1997). Using the same lectin binding methods, we compared the oligosaccharide moieties on authentic gradient-purified EBOV and the baculovirus expression products. Virion GP was found to react most strongly with *Datura stramonium* agglutinin (DSA), indicating the presence of complex or hybrid N-linked glycans. In addition, it reacted, albeit weaker, with *Arachis hypogaea* (peanut) agglutinin (PNA), which recognizes unsubstituted galactose β(1–3)N-acetylgalactosamine cores in O-glycans, and *Maackia amurensis* agglutinin (MAA) which reacts specifically with α(2–3)-linked sialic acid, demonstrating the presence of unsubstituted O-glycans and oligosaccharides containing sialic acid, respectively. The ZEBOV virion GP did not react with *Galantus nivalis* agglutinin (GNA), indicating the absence of high mannose N-linked glycans.

In contrast to ZEBOV virion GP, baculovirus-derived GP, either cell-associated or secreted, reacted most strongly with GNA, indicating modification primarily by high-mannose oligosaccharides. Similar to our observation with ZEBOV virion GP, the baculovirus-derived GP reacted weakly with PNA, denoting the presence of unsubstituted O-linked oligosaccharides. Baculovirus-derived GPs did not react with DSA, MAA, or *Sambucus nigra* agglutinin (SNA) (which reacts specifically with α(2–6)-linked sialic acid) indicating the absence of complex/hybrid N-linked glycans, or those containing sialic acid. Therefore, as demonstrated for MARV, the baculovirus derived EBOV GPs have both O-linked and N-linked carbohydrates, but these carbohydrates differ from those found in ZEBOV, and the differences could be the reason for the apparent size discrepancies of the insect-cell derived and mammalian cell-derived proteins.

To determine if the glycosylation differences or other modifications of the GP proteins expressed in insect cell cultures, affected antigenic changes we analyzed the recombinant proteins by immune-precipitation with a panel of monoclonal antibodies that recognize five distinct epitopes on GP_1 (Wilson et al., 2000). We found that all five antibodies precipitated the baculovirus-derived GP_1 from the $GP_{1,2}$ construct (Fig. 1D) or from the GPa- construct (data not shown). As described earlier for these monoclonal antibodies (Wilson et al., 2000), GP_2 was also precipitated from the $GP_{1,2}$ construct, presumably because it remains disulfide bonded to GP_1. No GP_2 was detected in cells infected with the GPa- construct (not shown), consistent with results presented in Fig. 1A. Epitopes bound by the antibodies in competition groups 1, 2 and 3 (Fig. 1D) recognize linear epitopes on GP_1, but those in groups 4 and 5 are conformational epitopes (Wilson et al., 2000). These studies suggest that the antigenic structure of the baculovirus-derived GPs remains intact.

To assess the immunogenicity of the baculovirus-derived EBOV proteins, we first performed a pilot vaccine study in which small groups of guinea pigs were vaccinated with various preparations of baculovirus-derived EBOV GP and GPa- proteins. We found that expression products from both the full-length and truncated GP genes elicited antibodies to GP and conferred partial protection from viral challenge (data not shown). These results were similar to those obtained with baculovirus-derived MARV proteins (Hevey et al., 1997). To determine if, as observed for MARV, a prime-boost combination of a DNA vaccine and the baculovirus-derived protein could provide better protective immunity than either vaccine alone in the guinea pig model, we vaccinated inbred guinea pigs and challenged them with ZEBOV.

The DNA vaccine or control plasmid were precipitated onto gold beads and loaded into plastic gene gun cartridges as previously described (Vanderzanden et al., 1998). Groups of six strain 13 guinea pigs received DNA only, baculovirus-derived ZEBOV $GP_{1,2}$ or GPa- only, or a combination of the two vaccines. For DNA vaccinations, guinea pigs received 2.5 μg of DNA at two inoculation sites delivered by the *PowderJect-XR* gene delivery device, as previously described (Vanderzanden et al., 1998). Guinea pigs that received baculovirus-derived antigens were vaccinated subcutaneously at two dorsal sites with a total volume of $\sim$0.5 ml (approximately 5 μg ZEBOV GP or GPa- per animal). A total of three vaccinations were administered at 4-week intervals. For the prime-boost vaccinations, groups of six guinea pigs received a priming dose of the DNA vaccine followed by two booster doses of the baculovirus products in adjuvant. A control group received a DNA vaccine consisting of an empty DNA plasmid. Blood samples were collected at the time of each vaccination and 2 weeks after the final vaccination. Serum antibody titers to purified ZEBOV were determined for each guinea pig by ELISA (Hevey et al., 1997; Vanderzanden et al., 1998) and geometric mean titers (GMT) for each vaccine group were calculated (Table 1). After the first vaccination, only guinea pigs receiving the $GP_{1,2}$ vaccine had detectable antibody responses to ZEBOV. After the second vaccination antibody responses were detected in all groups except the negative control group, and after the final vaccination, all groups had GMT by ELISA $\geq \log_{10}$ (data not shown).

Three weeks after the third vaccination, guinea pigs were challenged with 1000 PFU of guinea pig-adapted ZEBOV and observed daily for 28 days for signs of illness and mortality. Seven days after challenge, blood samples were collected from the guinea pigs and plaque

Table 1
Vaccination and challenge of guinea pigs

Prime vaccine	Boost vaccine	Log_{10} ELISA titer[a]	nAb/total[b] ($PRNT_{80}$)	Survivors/total[c]	Viremia[d] (viremic/total)	Mean day of death (range)
GP DNA	GP DNA	3.0	1/6 (1:40)	1/6	3.7 (5/6)	12 (10–18)
Control DNA	Control DNA	NA	0/6 (NA)	0/6	4.4 (6/6)	9 (8–10)
GP DNA	Baculo $GP_{1,2}$	3.7	1/6 (1:40)	0/6	2.7 (4/6)	12 (10–16)
GP DNA	Baculo GPa-	3.9	4/6 (1:80)	2/6	3.1 (3/6)	12 (10–13)
Baculo $GP_{1,2}$	Baculo $GP_{1,2}$	3.6	4/6 (1:40)	3/6	(0/6)	17 (14–19)
Baculo GPa-	Baculo GPa-	3.3	4/6 (1:60)	1/6	2.8 (3/4)[e]	8 (6–12)

[a] Geometric mean titers after three vaccinations.
[b] Number of guinea pigs that developed neutralizing antibodies after three vaccinations/total (nAb/T) and the dilution of serum that resulted in 80% plaque reduction in a neutralizing antibody test. Where ≥ 2 animals were positive an average was calculated.
[c] Survivors/total on Day 28 after s.c. challenge with 1000 PFU guinea pig adapted EBOV.
[d] Viremia (Log_{10} PFU/ml) 7 days after challenge. Where ≥ 2 animals were viremic, a geometric mean titer was calculated.
[e] Serum was collected from four surviving guinea pigs, the other two had already succumbed to infection.

assays were performed to measure viremia. All of the control guinea pigs had high viremias and died between eight and 10 days after challenge (Table 1). Five of the six DNA-vaccinated guinea pigs were viremic and died, although levels of viremia were slightly lower than observed in controls, and death was delayed in most animals (Table 1). The guinea pigs that received the baculovirus-derived $GP_{1,2}$ did not have detectable viremia at 7 days after challenge; however, all of these guinea pigs became sick later in the study and three of six died with a delayed mean time to death as compared to controls. The guinea pigs that received a priming dose of the DNA vaccine followed by two additional vaccinations with the baculovirus derived $GP_{1,2}$ or GPa- in adjuvant had reduced levels of viremia as compared to the controls; however, only two of the twelve guinea pigs, both in the GPa- group, survived the challenge. The time to death was also delayed for most of these guinea pigs (Table 1). All of the surviving guinea pigs showed increases in antibody levels when assayed by ELISA 44 days after challenge, indicating that all had been infected, and that sterile immunity was not achieved with any of the vaccines.

Neutralizing antibody responses to EBOV have been suggested to play a role in protective immunity (Jahrling et al., 1996; Maruyama et al., 1999b; Wilson et al., 2000), although correlation of neutralizing antibody levels and protection has not been reported. In our study, only one of the guinea pigs in the groups that received the DNA alone or the DNA followed by the $GP_{1,2}$, boost had detectable neutralizing antibodies, and neither survived the challenge. In the two groups that received the baculovirus proteins in adjuvant, four of six guinea pigs in each group had $PRNT_{80\%}$ titers of 1:40 or greater. However, there was no correlation with protection. For example, the lone survivor in the GPa- group did not have detectable neutralizing antibodies, and only one of three survivors in the $GP_{1,2}$, group had detectable neutralizing antibodies. The best neutralizing antibody responses were obtained in guinea pigs given the $GP_{1,2}$, DNA prime followed by the GPa- boost, with four of six animals having $PRNT_{80\%} \geq 1{:}40$. Nevertheless, there was little, if any correlation with protection. One of the two survivors had a reasonably high $PRNT_{80\%}$ titer (1:160), but the other survivor did not have detectable neutralizing antibodies.

The type of immune response needed to protect from filovirus challenge is not completely understood, however, it is likely that both strong cellular and humoral immune responses are needed. Generally, gene gun delivered DNA vaccines have been characterized as eliciting CD4+ Th2-type immune response in mice, as reflected by a predominant IgG1 antibody response. In contrast, intramuscular injection of a DNA vaccine elicits a mostly Th1-type response (Feltquate et al., 1997; Pertmer et al., 1996). This generalization has led to a feeling that gene gun delivery is better when the outcome desired is a strong antibody response than when a strong cell-mediated immune response is needed. However, we found that gene gun delivered EBOV DNA elicited cytotoxic T lymphocyte responses in mice (Vanderzanden et al., 1998). Measuring a cell-mediated immune response is more difficult in guinea pigs than mice, and the significance of indirect measures, such antibody isotypes, which correlate with Th1-like or Th2-like responses in mice, are not defined for guinea pigs.

Consequently, as a measure of the ability of the baculovirus-derived proteins to elicit cell-mediated immune responses, we evaluated the T-cell stimulatory capacity of the baculovirus-expression products in cultured human dendritic cells and monocytes as described previously (Saikh et al., 2001). Briefly, dendritic cells or monocytes were incubated with autologous T cells and supernatants from baculovirus-infected cell cultures containing EBOV GPa-. These T cells were then used as effector cells in a cytotoxicity assay using HLA-matched or -unmatched B cell target cells that had been pulsed with supernatants from baculovirus infected cell cultures containing EBOV GPa-, an unrelated viral antigen, or no antigen. Although we assayed both $GP_{1,2}$

Table 2
T-cell stimulatory capacity of GPa- in cultured human dendritic cells[a]

	Percent cytotoxicity			
	DC activated CTL		Monocytes	
	Effector(T cell):target (BSM cell)[b]			
	1:1	10:1	1:1	10:1
Targets (Experiment 1)				
BSM, no antigen[c]	30	26	26	28
BSM+GPa-	29	53	16	38
BSM+control supernatant	12	2	0	2
K562[d]+GPa-	8	24	3	18
Targets (Experiment 2)[e]				
BSM, no antigen	15	20	N/D[f]	N/D
BSM+Gpa-	30	64	N/D	N/D
BSM+control antigen	11	12	N/D	N/D

[a] Adherent monocytes from a HLA-A2 donor were cultured with IL-15 for 60 h to transform them into dendritic cells (DC). Cells were washed extensively to remove IL-15 and nonadherent cells. Monocytes cultured without IL-15 remain as monocytes throughout culture. Autologous T cells and Antigens (10 μg/ml) were added to the culture. After 6 days, T cells were collected and used as effector cells in cytotoxicity assays.

[b] Effector cell (T cell) to target cell (BSM cell) ratio.

[c] BSM (B cell, HLA-A2) were pulsed with Ebola Gpa- (10 μg/ml) culture supernatants or supernatants from cells infected with a wild-type baculovirus (control in Experiment 1), or culture supernatant from a baculovirus expressing an unrelated protein (Experiment 2) for 1 h at 37 °C and used as target cells.

[d] non-HLA matched control target cells (K562 cells).

[e] Experiment 1 and Experiment 2 are similar assays except that DC-activated effector CTLs for experiment 1 were made by culturing autologous T cells with DC and Ebola antigen wheras in experiment 2 autologous T cells were cultured with DC infected with a VEE replicon expressing EBOV GP.

[f] Not determined (N/D).

and GPa- concentrated from insect cell culture supernatants, the background reactivity of the $GP_{1,2}$ preparations was too high to achieve valid results. This is likely due to the lower concentration of the expression product in the supernatant as compared to that in supernatants from cells infected with the GPa- baculovirus.

In two independent assays performed with the GPa- expression product, we observed zero to twofold increases in cytotoxicity in HLA-matched cells when we used an effector:target ratio of 1:1 (Table 2). When the effector:target ratio was increased to 10:1, a twofold increase in cytotoxicity was measured in one experiment and a greater than threefold increase was seen in the second experiment. No increase in cytotoxicity was seen in pulsed HLA-unmatched cells or with a control antigen at either effector:target ratio. Monocyte activated CTLs were also evaluated in one of the assays and revealed that EBOV GPa- induced a 1.4-fold increase in cytotoxicity over background at an effector:target ratio of 10:1. An enhanced cytotoxicity of monocyte activated CTLs was not seen at the 1:1 effector:target ratio (Table 2). These data provide evidence that the baculovirus derived GPa- expression product could potentially elicit not only humoral responses, but also cell-mediated responses to EBOV. These results, indicate that the baculovirus-derived proteins are able to elicit cytotoxic T cell responses in human cells, and presumably also in guinea pigs.

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